

## 1. PRODUCT AND COMPANY INFORMATION

### 1.1 Product Identifiers

Product Names: ZW800-1 Forte-Tetrafluorophenyl (TFP)

Product Numbers:

CAS-No.: N/A

### 1.2 Product Information

N-Hydroxysuccinimide (NHS or –OSu) activated esters are commonly used in bio-conjugation protocols because they are so versatile and easy to use. Activation of the carboxylic acid enables the formation of a stable covalent amide bond to form, making these reagents ideal for one-step conjugation to primary amino groups of small molecules, peptides, antibodies, proteins, and polymers. However, an unfortunate side reaction that occurs is the rapid hydrolysis of the NHS ester to the non-reactive carboxylic acid in the required basic conditions. Tetrafluorophenyl (TFP) esters allow for the same reactivity to amines as NHS esters with a significantly slower rate of hydrolysis, representing a significant improvement over NHS esters. TFP esters allow for the same easy, one-step conjugation reactions to free amines as the NHS but with a much slower rate of hydrolysis.

Labeling the amino groups of small molecules, peptides, and peptidomimetics using a TFP ester is typically performed in mildly basic anhydrous solvent while at room temperature. However, the labeling of biomolecules needs to be performed in an aqueous environment, in which case hydroxyl ions from water can act as a nucleophile, forming the unreactive carboxylic acid as a byproduct. In these cases, the use of a TFP activated starting material allows for superior conversion of starting material to product and less hydrolysis to the carboxylic acid than would be the case for the NHS derivative. In aqueous environments, TFP esters represent a vast improvement over the equivalent NHS ester.

In addition to pH, and assuming that the buffer used does not contain any extraneous primary amines (aside: never use Tris buffer for an TFP labeling), the most critical parameter is the rate of the reaction. Just like in any chemical reaction, the rate (corresponding to completeness of the reaction for a given length of time) is proportional to the product of the two reactants, in this case the primary amine and the TFP ester. In general, approximately 1 mM of each reactant will lead to completion of the reaction. And, the higher the concentration of each the better, with the upper limit only dependent on solubility of the reactants in the chosen buffer. Remember, too, that the concentration of available epsilon amines in a protein depends on how many lysines are present. For example, a protein with 10 lysines used at a concentration of 100  $\mu$ M is actually 1 mM in primary amine, and thus will usually drive the reaction to completion (assuming all lysines are surface accessible).

ZW800-1 Forte-TFP is an improvement over ZW800-1 Forte-NHS that allows for the same amide bond formation in a simple, one-step conjugation reaction with a much lower rate of hydrolysis in aqueous environments. This allows for more robust reaction conditions with less starting material being required to overcome hydrolysis to the unreactive carboxylic acid.

### 1.3 Details of the supplier for the Instructions for Use

Company: Curadel ResVet Imaging, LLC  
257 Simarano Drive, Suite 100  
Worcester, MA 01605  
USA

Telephone: 1 508 305 2355

Fax: 1 508 251 2029

E-mail: [support@resvet.curadel.com](mailto:support@resvet.curadel.com)

URL:	<a href="http://www.curadelresvetimaging.com">www.curadelresvetimaging.com</a>
<b>1.4 Emergency Telephone Number</b>	
Emergency Phone #:	1 508 305 2355

## 2. HANDLING

<b>Precautions</b>	<p><b>This contrast agent is intended for laboratory research use only. Not for diagnostic procedures. Not for veterinary or human use.</b></p> <p>Wear appropriate protective equipment including laboratory coat, gloves, and eyewear. Avoid contact with skin, eyes, and mouth. Contrast agent will stain clothing and skin.</p>
<b>Preparation</b>	<p>Prepare a stock solution of ZW800-1 Forte-TFP in anhydrous DMSO.</p> <p><b>Sterile Use:</b> Whenever sterile use is intended, for example, intravenous injection into a research animal or addition to cell culture medium, filter the final NIR fluorophore solution through a 0.22 µm filter.</p>
<b>Storage</b>	<p>Lyophilized powder can be stored at room temperature up to one year. Protect from light. Stock solutions should be kept at 4-8 C when in DMSO. Stock solutions in water should be kept at -20 C. Avoid excessive freeze/thaw cycles by aliquoting solution prior to freezing.</p> <p>Discard stock solutions after 3 months when stored properly. Discard stock solutions after 1 week when stored at room temperature.</p>

## 3. USAGE

<b>Small Molecule Conjugation</b>	<p>Prepare a stock solution of ZW800-1 Forte-TFP as described in the preparation section above. Add to the desired small molecule with a free amine in a ratio between 1.1-2 molar equivalents of the small molecule to ZW800-1 Forte-TFP dissolved in anhydrous DMSO. Add between 3-10 equivalents of N,N-Diisopropylethylamine (exact amount required will depend upon the pKa of the small molecule, other bases can be used as required but it is best to ensure any amine containing bases act as poor nucleophiles) to the reaction mixture and let this react at room temperature for 0.5-2 hours with constant agitation. If the reaction ceases to progress, add an additional equivalent of base and monitor after 15 minutes. Continue this until the reaction is complete. If the reaction still does not go to completion, add an additional equivalent of the small molecule. The easiest way to monitor the reaction is to use a liquid chromatographic system (equipped with C18 column) with PDA detector, monitoring at 660 nm.</p>
<b>Large Molecule Conjugation</b>	<p>Prepare a stock solution of ZW800-1 Forte-TFP as described in the preparation section above. Add to the desired large molecule (prepared in a buffered aqueous solution) a ratio between 1.1-2 molar equivalents of ZW800-1 Forte-TFP. Add the required amount 1M sodium hydroxide in water to the reaction mixture to achieve a pH of approximately 8. Let this react at room temperature for 0.5-2 hours with constant agitation. If the reaction ceases to progress, add an additional equivalent of ZW800-1 Forte-TFP. Continue this until the reaction is complete. If the reaction still does not progress confirm the pH is still 8 or greater and adjust as necessary. The easiest way to monitor the reaction is to use a liquid chromatographic system (equipped for size exclusion chromatography) with PDA detector, monitoring at 755 nm.</p>